

Title: Snord116 is critical in the regulation of food intake and body weight

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Supplementary Figure 1

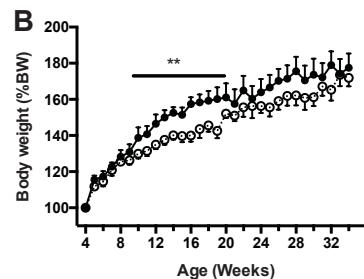
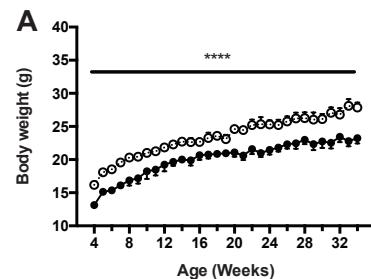
Low body weight, increased growth rate in early stage, altered body composition, and increased energy intake and energy expenditure in female Snord116^{-/-} mice (—●—; black bars) vs. control mice (···○···; white bars) on chow diet.

A) Absolute weekly body weight from 4 to 35 weeks of age; **B)** growth rate normalised to body weight (BW) at 4 weeks of age;

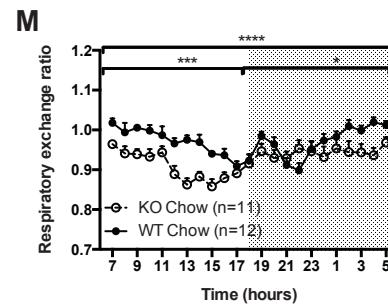
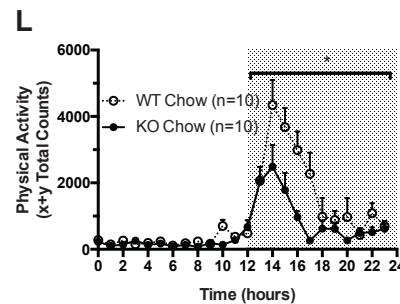
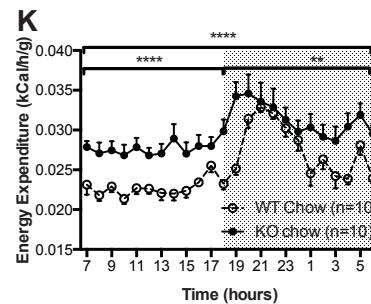
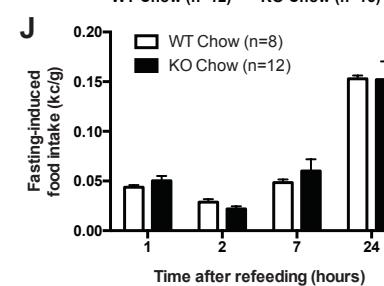
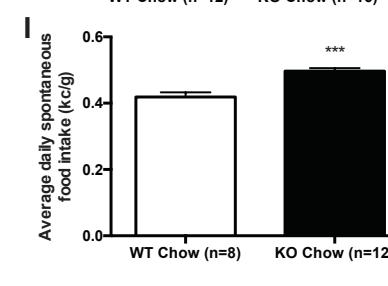
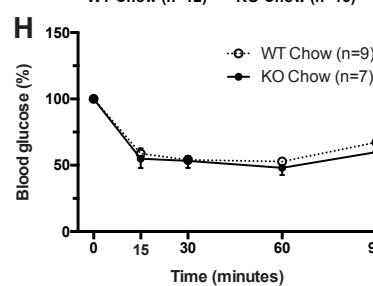
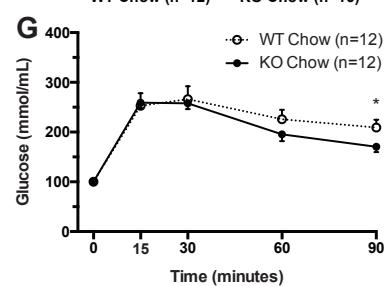
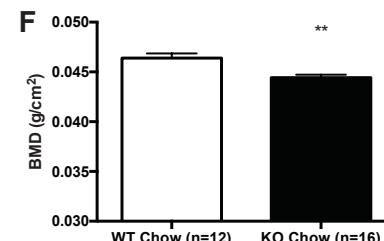
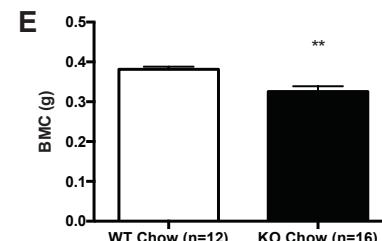
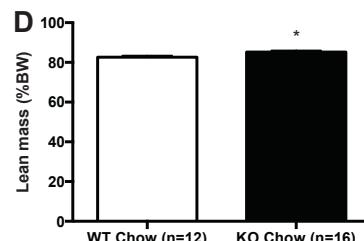
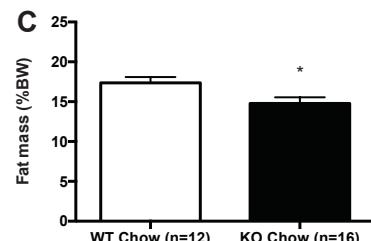
In early adulthood (12-16 weeks of age): **C)** fat mass as a percentage of BW (%BW); **D)** lean mass (%BW); **E)** bone mineral content (BMC); **F)** bone mineral density (BMD); **G)** blood glucose curves during intraperitoneal glucose tolerance test as a percentage of fasting glucose levels (GTT); **H)** blood glucose curves during insulin tolerance test as a percentage of fasting glucose levels (ITT); **I)** spontaneous 24-hour calorie intake (%BW), expressed as the average of triplicate readings over three consecutive days; **J)** fasting-induced calorie intake (%BW); **K)** 24-hour energy expenditure; **L)** 24-hour physical activity; **M)** 24-hour respiratory exchange ratio (RER).

In late adulthood (28-32 weeks of age): **N)** fat mass (%BW); **O)** lean mass (%BW); **P)** BMC; **Q)** BMD; **R)** GTT; **S)** ITT; **T)** spontaneous 24-hour calorie intake (%BW); **U)** fasting-induced calorie intake (%BW); **V)** 24-hour energy expenditure; **W)** 24-hour physical activity; **X)** RER.

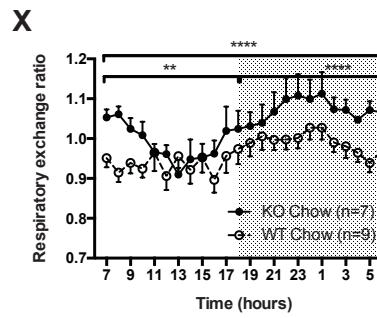
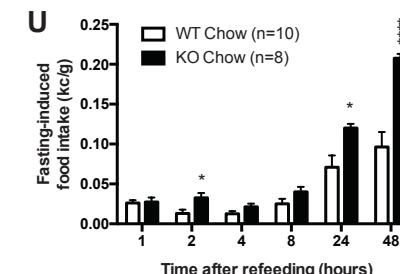
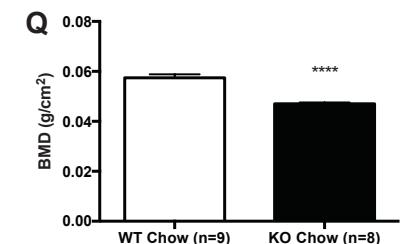
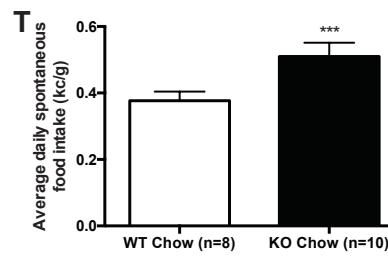
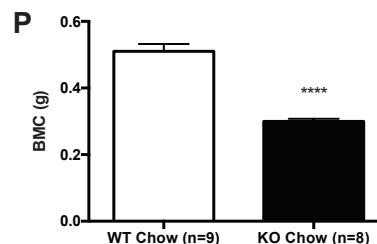
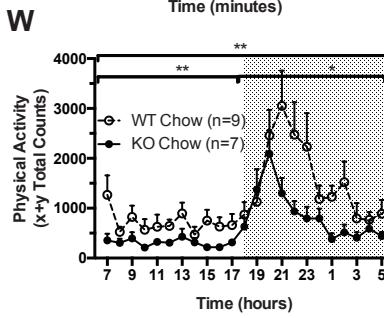
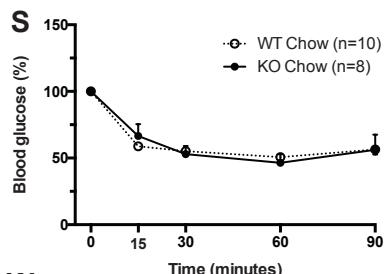
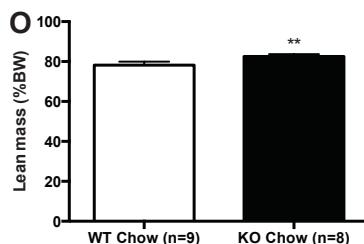
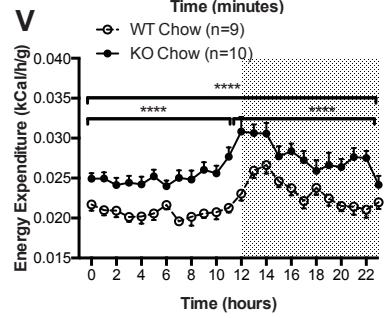
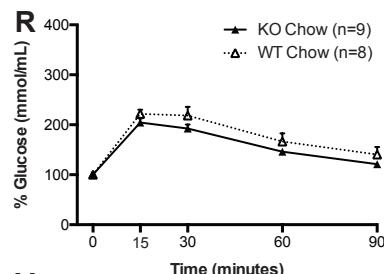
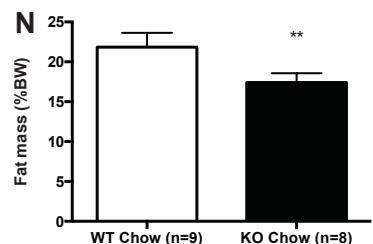
(* P<0.05, ** P<0.01, *** P<0.001, and **** P<0.0001)



Early adulthood



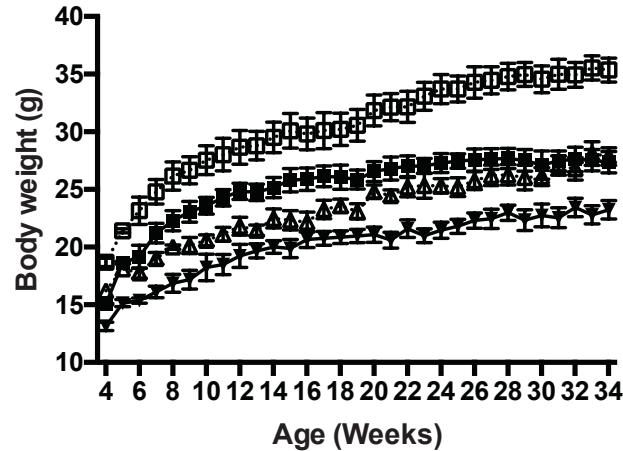
Late adulthood



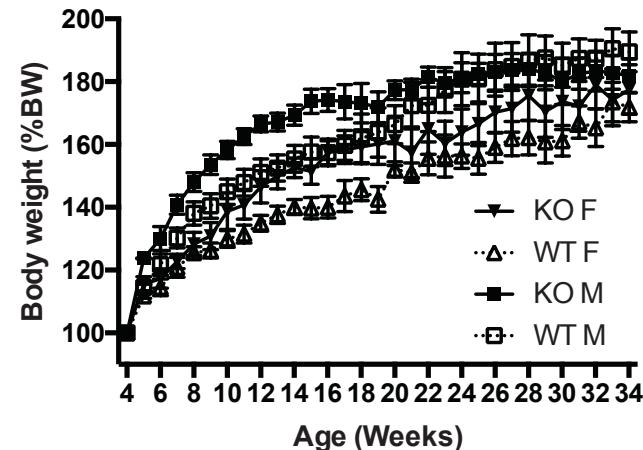
Supplementary Figure 2

Male-and-female combined graphs of absolute weekly body weight (**A**), body weight gain (**B**) and food intake (**C**) and the statistic comparisons between groups (**D**) showing the significant difference between genotypes ($\text{Snord116}^{-/-}$ and the wild type) and genders (male and female).

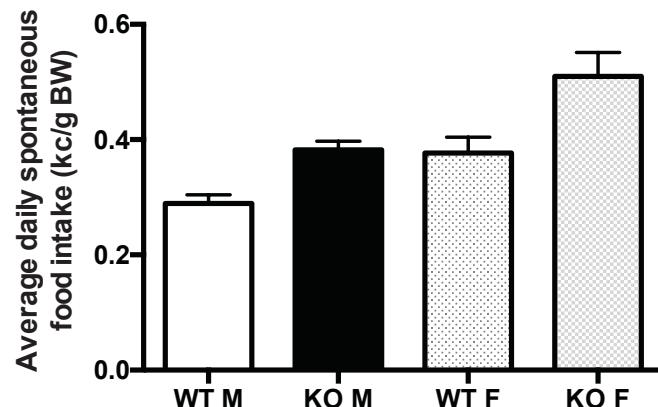
A



B



C

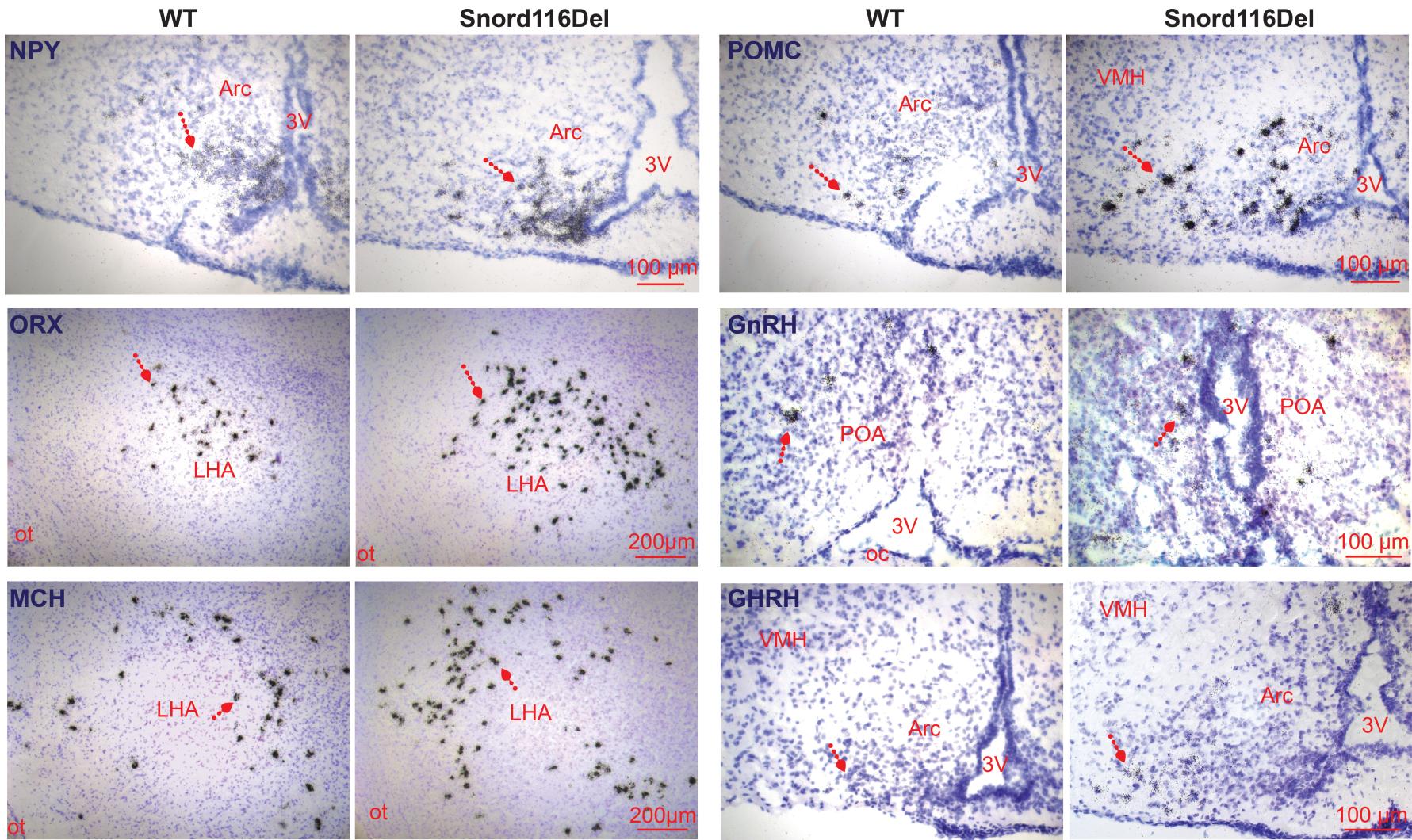


D

Tukey's multiple comparisons test	Body weight significant	Body weight gain (Week 6 to 18; %BW) significant	Food intake significant
WT M vs. KO M	***	***	***
KO M vs. KO F	**	***	**
WT F vs. KO F	*	**	*
WT M vs. WT F	**	****	ns

Supplementary Figure 3

Expression of appetite-related neuropeptides in hypothalamic nuclei, detected by *in situ* hybridisation (black dots indicated by red arrows; left panels: wildtype (WT), right panels: Snord116^{-/-}; neuropeptide Y (NPY), orexin (ORX), melanin concentrating hormone (MCH), proopiomelanocortin (POMC), gonadotropin-releasing hormone (GnRH), growth hormone-releasing hormone (GHRH), third ventricle (3V), arcuate nuclei (ARC), lateral hypothalamic area (LHA), optic chiasm (oc), optic tract (ot), preoptic area (POA), ventromedial nuclei (VMH)).



Supplementary table 1:

Gene set enrichment analysis (GSEA) from the collection of gene sets derived from the Biological Process Ontology revealed that 463 out of 569 gene sets were down- regulated in KO with 85 gene sets significant at FDR <25% (Table 1). Out of the remaining 106 gene sets which were upregulated in KO, only 1 gene set was significantly enriched at FDR <25% (Table 2).

Name	Size	ES	NES	Nom p-val	FDR q-val
Regulation_of_nucleocytoplasmic_transport	21	0.657	1.956	0.000	0.068
Regulation_of_intracellular_transport	24	0.610	1.923	0.000	0.069
Feeding_behavior	24	0.594	1.915	0.000	0.046
Negative_regulation_of_transport	19	0.589	1.841	0.000	0.066
Cytokine_production	66	0.477	1.825	0.000	0.062
Carbohydrate_transport	18	0.647	1.824	0.000	0.052
Regulation_of_cytokine_production	23	0.586	1.778	0.000	0.090
Fatty_acid_metabolic_process	60	0.481	1.776	0.000	0.079
Positive_regulation_of_transport	21	0.590	1.771	0.015	0.078
Positive_regulation_of_cell_differentiation	24	0.563	1.767	0.000	0.071
Regulation_of_myeloid_cell_differentiation	17	0.598	1.753	0.014	0.078
Skeletal_development	97	0.426	1.745	0.000	0.079
Rna_processing	152	0.386	1.736	0.000	0.086
Cellular_defense_response	45	0.494	1.730	0.000	0.089
Positive_regulation_of_multicellular_organismal_process	61	0.437	1.725	0.000	0.091
Protein_targeting	102	0.407	1.722	0.000	0.090
Meiosis_i	20	0.581	1.714	0.000	0.091
Regulation_of_protein_import_into_nucleus	15	0.638	1.713	0.000	0.086
Protein_import_into_nucleus	44	0.480	1.709	0.000	0.087
Regulation_of_transport	64	0.445	1.707	0.000	0.084
Establishment_of_protein_localization	178	0.381	1.706	0.000	0.080
Regulation_of_gene_expression_epigenetic	29	0.521	1.704	0.000	0.079
Meiotic_cell_cycle	34	0.502	1.702	0.014	0.078
Coenzyme_metabolic_process	37	0.487	1.697	0.000	0.078
Nuclear_transport	81	0.414	1.685	0.000	0.085
Nucleocytoplasmic_transport	80	0.417	1.684	0.000	0.083

Protein_localization	201	0.366	1.680	0.000	0.080
Regulation_of_multicellular_organismal_process	140	0.389	1.678	0.000	0.079
Innate immune_response	18	0.601	1.675	0.018	0.079
Nuclear_import	46	0.472	1.669	0.000	0.079
Activation_of_immune_response	17	0.558	1.667	0.016	0.078
Dna_recombination	46	0.441	1.666	0.000	0.076
Regulation_of_cell_differentiation	57	0.444	1.663	0.000	0.075
Reproductive_process	136	0.389	1.649	0.000	0.086
Protein_import	58	0.442	1.649	0.000	0.084
Icosanoid_metabolic_process	15	0.599	1.646	0.000	0.083
Lipid_metabolic_process	299	0.353	1.645	0.000	0.082
Protein_secretion	29	0.510	1.644	0.042	0.081
Monocarboxylic_acid_metabolic_process	83	0.417	1.642	0.000	0.080
Organic_acid_metabolic_process	171	0.367	1.618	0.000	0.103
Regulation_of_immune_response	32	0.497	1.617	0.000	0.103
Cofactor_metabolic_process	52	0.449	1.614	0.000	0.103
Carboxylic_acid_metabolic_process	169	0.371	1.612	0.000	0.103
Regulation_of_protein_secretion	19	0.543	1.611	0.017	0.101
Immune_effector_process	32	0.490	1.598	0.000	0.112
Macromolecule_localization	221	0.364	1.598	0.000	0.109
Regulation_of_immune_system_process	61	0.421	1.589	0.000	0.115
Intracellular_protein_transport	136	0.377	1.578	0.000	0.126
Regulation_of_response_to_stimulus	54	0.437	1.577	0.000	0.125
Cytokine_secretion	15	0.578	1.562	0.016	0.141
Bone_remodeling	26	0.474	1.553	0.031	0.149
Protein_transport	147	0.356	1.546	0.000	0.153
Adaptive immune_response_go_0002460	23	0.473	1.537	0.015	0.163
Multi_organism_process	131	0.359	1.531	0.000	0.168
Sensory_perception	181	0.345	1.529	0.000	0.167
Cation_homeostasis	96	0.355	1.527	0.000	0.167
Tissue_remodeling	27	0.481	1.522	0.030	0.171
Female_pregnancy	38	0.425	1.521	0.015	0.169
Positive_regulation_of_immune_response	28	0.488	1.514	0.066	0.173
Regulation_of_binding	57	0.405	1.511	0.013	0.174
Protein_homooligomerization	21	0.508	1.503	0.076	0.182
Immune_system_development	76	0.378	1.501	0.000	0.182

Defense_response	224	0.322	1.487	0.012	0.198
Tissue_development	130	0.349	1.486	0.000	0.198
Viral_reproduction	37	0.431	1.478	0.015	0.210
Meiotic_recombination	17	0.532	1.475	0.045	0.211
Homophilic_cell_adhesion	16	0.534	1.474	0.046	0.211
Negative_regulation_of_multicellular_organismal_process	29	0.468	1.471	0.043	0.213
Positive_regulation_of_immune_system_process	47	0.408	1.470	0.039	0.211
Rna_splicing	80	0.374	1.469	0.000	0.210
Adaptive immune_response	24	0.481	1.469	0.057	0.208
Amino_acid_metabolic_process	74	0.367	1.469	0.042	0.205
Hemopoietic_or_lymphoid_organ_development	73	0.376	1.468	0.000	0.204
Cellular_lipid_metabolic_process	236	0.325	1.466	0.012	0.204
Hemopoiesis	71	0.373	1.461	0.014	0.210
Inflammatory_response	115	0.344	1.459	0.013	0.210
Dna_repair	121	0.345	1.458	0.024	0.208
Regulation_of_muscle_contraction	18	0.476	1.455	0.063	0.211
Nucleobasenucleoside_and_nucleotide_metabolic_process	50	0.387	1.453	0.081	0.213
Protein_oligomerization	39	0.414	1.452	0.055	0.213
Positive_regulation_of_response_to_stimulus	38	0.421	1.440	0.069	0.228
Immune_response	207	0.319	1.440	0.000	0.226
Positive_regulation_of_caspase_activity	29	0.444	1.438	0.044	0.227
Anatomical_structureFormation	54	0.385	1.437	0.038	0.225

Supplementray Table 2: DNA oligonucleotide sequence of neuropeptides for *in situ* hybridisation

Neuropeptide	DNA oligonucleotide sequence
mouse Snord116	5'-GTTCAGCTTTCCAAGGAATGTTGACTGGGAATCATCATAGATCC-3'
mouse GHRH	5'-GCTTGCTCTGTCCACATGCTGCTTCCTGGCGGCTGAGCCTGG-3'
mouse NPY	5'-GAGGGTCAGTCCACACAGCCCCATTGCTTACCTAGCAT-3'
mouse POMC	5'-TGGCTGCTCTCCAGGCACCAGCTCACACATCTATGGAGG-3'
mouse MCH	5'-TTTCCTGTGTGGACTCAGCATTCTGAACCTCATTCTCAGCTGG-3'
mouse orexin	5'-CTTCCCAGAGTCAGGATAACCGCAGCGTGGTGCAGCTCCGTGC-3'
mouse GnRH	5'-CAAACACACAGTCAGCAGTAGAATGCCGGCCATCAGTTGAGGATC-3'
mouse oxytocin	5'-TCCGCGCAGCAGATGCTTGGTCCGAAGCAGCGTCCTTGCCGC-3'
mouse TH	5'-AACCTTACTCCTCCAGAGGTTCCCTGACCCAGGCTTCCAGTTGTG-3'